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## MARINE BACTERIA AND LOCALIZED CORROSION ON POLYMER COATED STEEL: CAUSE AND EFFECT

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### ABSTRACT

Diagnosis of microbiologically influenced corrosion on iron-containing substrata exposed in marine environments cannot be based solely on spatial relationships between large accumulations of bacterial cells and iron corrosion products. Field experiments were designed to evaluate the relationship between marine bacteria and localized corrosion on coated mild steel. In all cases, the distribution of bacteria was strongly influenced by the presence of iron corrosion products independent of coating combinations. In the presence of cathodic protection, coating defects were filled with calcareous deposits and few bacterial cells. Results demonstrate that bacteria are preferentially attracted to iron corrosion products in coating defects and that attraction is more influential than topography in determining the spatial distribution of bacterial cells.

**KEYWORDS:** cathodic polarization, marine bacteria, seawater, mild steel, polymer coatings

### INTRODUCTION

The role that bacteria play in producing/influencing anodic and cathodic corrosion reactions has been documented in the literature on microbiologically influenced corrosion (MIC). However, the impact of anodic and cathodic polarizations on deposition or settlement of bacteria has not been adequately addressed. One of the most serious challenges in identification of MIC is differentiation between biological and abiological processes. Most MIC investigations determine spatial relationships between bacteria and corrosion products using microscopy or microbiological diagnostic kits. Spatial relationships are then interpreted as causal. The reasoning is that because some number of microorganisms traditionally associated with MIC (i.e., sulfate-reducing bacteria {SRB}, metal-depositing or metal-reducing bacteria) and corrosion products are co-located, the microorganisms must have caused the corrosion. Experiments have been designed to evaluate spatial relationships between corrosion products and marine bacteria. Crevices were allowed to form on 304 stainless steel in abiotic seawater with accumulations of corrosion products.<sup>[1]</sup>

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Marine bacteria were added to the medium and allowed to remain in contact with the corroding specimen for 14 days. At the end of the exposure period, microscopy was used to determine the location of marine bacteria. In all cases, large concentrations of cells were associated with abiotically formed iron corrosion products. The organisms did not cause the corrosion, but were co-located with the iron corrosion products. Interpretation of spatial relationships on polymer coated materials is further complicated because bacteria preferentially colonize surface inhomogeneities including scratches, cracks, and pinholes.<sup>[2,3]</sup> These same areas are the first to exhibit localized corrosion in abiotic, artificial seawater.

In an attempt to determine the relative importance of topography and iron corrosion products in the spatial distribution of marine microorganisms, field experiments were designed to evaluate relationships between marine bacteria and surface defects in a series of polymer coatings (Table 1) on mild steel. The coatings differed in composition of primer, midcoat, topcoat, and coating thickness. Specimens were exposed with and without zinc anodes to provide cathodic protection. Environmental scanning electron microscopy/energy dispersive x-ray spectrometry (ESEM/EDS) were used to document the presence of cells and determine elemental composition of corrosion products, respectively.

## MATERIALS AND METHODS

### Panels

Triplicate cold rolled steel panels (10.16 cm × 15.24 cm) were coated with the following combinations: zinc primer and epoxy polyamide topcoat; zinc primer, epoxy polyamide midcoat, and polyurethane topcoat; phosphate primer and epoxy polyamide topcoat; phosphate primer, epoxy polyamide midcoat, and polyurethane topcoat; ion vapor-deposited (IVD) aluminum (Al) primer and epoxy polyamide topcoat; IVD-Al primer, epoxy polyamide midcoat, and polyurethane topcoat; and three layers of epoxy polyamide (Table 1). Panels were exposed with and without intentional defects. Defects were introduced by drilling four 0.32-cm-diameter holes (two per side) 2.54 cm from the bottom edge and 2.54 cm from the side edge (Fig. 1) through the coating to the metal surface to create a defined holiday in the coating. Some panels with intentional defects were cathodically protected by attaching a zinc anode; others were allowed to freely corrode.

### Seawater Exposures

Panels were exposed for 30 d to natural seawater at the Key West, FL, Naval Research Laboratory Marine Corrosion Facility and to artificial seawater in laboratory tests. At the end of exposure, panels from the natural seawater exposures were shipped to the Naval Research Laboratory, Stennis Space Center, MS (NRLSSC) in closed, Plexiglas® containers filled with natural seawater. Containers were constructed with slots so that multiple panels were secured in the orientation in which they had been exposed to prevent disruption of surface layers. Plexiglas® containers were shipped by overnight courier in insulated cartons with ice packs to slow biological activity.

### Photodocumentation

Upon arrival at NRLSSC, wet panels were photographed and examined with a Wild Heerbrugg M8 zoom stereo microscope. Areas of interest, including bubbles, holidays, delamination, blisters, corrosion, stains, etc., were photographed with a Polaroid MicroCam attachment using Polaroid type 339 color print film. Panels from the natural seawater exposures were transferred to separate containers of 4% glutaraldehyde in 0.45 µm filtered natural seawater as appropriate and refrigerated overnight. Mild steel panels from laboratory exposures were also transferred to a separate container of 4% glutaraldehyde in artificial seawater and refrigerated overnight. Prior to microscopic examination, each panel was removed from the glutaraldehyde fixative in seawater and gently rinsed in distilled water.

After photography and prior to ESEM examination, areas of interest were carefully cut from wet panels. Smaller wet sections were transferred to a refrigerated Peltier cooling device maintained at 4°C and imaged in a water vapor environment at 4–5 torr in a partially hydrated state. ESEM operating parameters have been described elsewhere.<sup>[4]</sup>



## RESULTS

### Seawater Exposures

Metallic zinc and IVD-Al primers (coatings A, B, E, and F) provided cathodic protection for coatings without intentional defects. After 30 days, these coatings were discolored due to the presence of microalgae and macrofouling, but there was no localized corrosion. In the absence of intentional defects, localized corrosion was prominent on surfaces with phosphate primer and the all-epoxy polyamide coatings (coatings C, D, and G). In all cases, delamination was accompanied by localized corrosion and accumulations of bacteria (Fig. 2). In the presence of cathodic protection by an external zinc anode, intentional defects were filled with calcareous deposits (see for example Fig. 3a-c). The EDS spectrum of the calcareous deposits indicates the presence of zinc and phosphate from the primer in addition to calcium. There were no indications of corrosion and the numbers of individual bacterial cells within the defects were similar to the numbers of cells on the intact coating. There were no accumulations of bacteria within any of the defects protected by an external anode.

Coating composition influenced corrosion behavior and location of bacteria in defects without cathodic protection from zinc anodes. Defects in the coatings with zinc primer were, in all cases, filled with calcareous deposits and no corrosion products or accumulations of bacteria were observed. While the zinc primer on steel provided cathodic protection to exposed steel in defects, the same effect was not consistently observed for samples with IVD-Al primer. One of the four panels with IVD-Al primer had no indication of corrosion products in any of the defects, while all others were filled with corrosion products. When corrosion was present, there were accumulations of bacterial cells.

In the absence of cathodic protection provided by an external anode or the primer, most defects in the other coatings were filled with voluminous corrosion products (Fig. 4a-c) and large accumulations of bacteria (Fig. 5a-c). ESEM images were collected from the center of the corroding defect. Large accumulations of bacteria could only be located in association with corrosion products. Even though the specimens were exposed to the same body of seawater, there were morphological differences among the bacteria within defects of various coatings.

## DISCUSSION

For the following discussion, the term "coating" will be used to refer to combinations of layers - primer, midcoat, and topcoat. Individual layers will be referred to as "coats." Coatings and cathodic protection are both engineering techniques with the primary purpose of mitigating or preventing corrosion. Cathodic protection reduces corrosion rates by shifting the potential of the corroding material to values where the rate of anodic metal dissolution is reduced. Coatings can be used in combination with cathodic protection. Cathodic potentials promote formation of hydroxyl ions on cathodic areas that forces a shift in the equilibria of chemical reactions involving calcium, magnesium, and bicarbonate ions so that calcium carbonate and magnesium hydroxide are formed in coating breaches.

Primer is the base coat on which the rest of the coating system is applied. Metallic aluminum and zinc can be used as primers to provide corrosion resistance. The inorganic matrix of the primer is conductive and allows aluminum or zinc to go into solution in a controlled manner, making either anodic to the steel and, thus, able to protect breaks in the coating. Eventually holidays, pinholes, scratches, or scars heal by the formation of reaction products, such as zinc hydroxide or calcium carbonate. Intermediate or mid-coats provide additional thickness that acts as a barrier from the environment, increased coating electrical resistance, as well as bonding between topcoat and primer. Topcoats provide resistant seals for the coating, an initial barrier to the environment, and a pleasing appearance.

In the marine exposures, rust layers were accompanied by large concentrations of marine bacteria irrespective of coating composition. There were no indications of corrosion within coating defects that had zinc primer with or without external anodes. In the absence of an anode, defects on samples A and B were filled with crystalline material (Fig. 3a-c) that was predominantly calcium. In the presence of an attached sacrificial zinc anode, defects in all coatings were filled with crystalline material that was predominantly calcium at all locations. Relative amounts of zinc and calcium varied. Furthermore, large concentrations of bacteria could not be detected within defect areas

protected by either zinc as primer or as sacrificial anode. In all other cases, defects were mounded with bright reddish-orange corrosion products (Fig. 4) and large concentrations of marine bacteria (Fig. 5). Tightly coiled helical shapes, typical of some iron-oxidizing bacteria, reportedly rare in the marine environment<sup>[5]</sup>, were concentrated within the corrosion products on samples C and D with phosphate primer, epoxy polyamide midcoat, and polyurethane topcoat (Fig. 5b). These organisms, in association with mounds or tubercles of iron corrosion products, are often used as indicators of MIC.<sup>[6,7]</sup>

Other investigators have observed the co-location of bacteria and anodic processes. Franklin et al. detected bacterial cells associated with anodic regions of carbon steel using scanning vibrating electrode microscopy.<sup>[8]</sup> They were cautious not to interpret their data to indicate that the cells caused the anodic sites. They did observe that once bacteria were associated with anodic regions, those areas remained anodic for the duration of the experiments. Anodic sites not associated with bacteria were transient. Similar observations of spatial relationships between *Oceanospirillum* and anodic regions on copper surfaces were made.<sup>[9]</sup> DeSanchez and Schiffrin demonstrated chemotaxis of a marine *Pseudomonas* sp. induced by transition metal ion concentration gradients from corroding copper and titanium surfaces.<sup>[10]</sup> Loeb and Neihof used microelectrophores to demonstrate that particles in the marine environment, including bacteria, are negatively charged and attracted to positive charge.<sup>[11]</sup>

In our experiments, cathodic protection did not influence the concentration of bacteria within intentional defects. Numerous investigators have demonstrated a relationship between marine fouling and calcareous deposits on cathodically protected surfaces.<sup>[12,13]</sup> The literature on the relationship between bacteria and cathodic protection is confusing and difficult to compare because of differing experimental conditions (laboratory vs. field) and differing techniques to evaluate the constituents within the biofilm. It has been reported that cathodic protection retards microbial settlement because of the alkaline pH generated at the surface. Dhar<sup>[14]</sup> demonstrated that a potential more negative than  $-0.95$  V (Cu/CuSO<sub>4</sub>) was effective in decreasing bacterial content on titanium in seawater by 1 to 2 orders of magnitude.

Licina<sup>[15]</sup> and Angell<sup>[16]</sup> independently developed techniques for measuring currents sustained by bacteria that require cathodic polarizations to "encourage biofilm formation."<sup>[15]</sup> Nekoksa and Gutherman<sup>[17]</sup> and Guezennec<sup>[18]</sup> showed that more marine bacteria settled on cathodically protected metals than on unpolarized ones. Gomez de Saravia<sup>[19]</sup> and Videla<sup>[20]</sup> demonstrated that cathodic polarization in a seawater medium increased numbers of SRB and decreased numbers of aerobic bacteria on carbon steel surfaces relative to unpolarized controls. They hypothesized that preferential increases or decreases in cell types were due to differences in cell isoelectric points.

## SUMMARY AND CONCLUSIONS

The distribution of bacteria on coated mild steel with defined defects was strongly influenced by the presence of iron corrosion products independent of coating composition. In natural marine exposures, bacteria were consistently co-located with iron oxide corrosion products. The nature of the attraction has not been identified, but may be related to chemotaxis and/or surface charge. When either a zinc primer or a zinc anode provided cathodic protection, defects were filled with calcareous deposits and contained only isolated bacterial cells, similar to areas of intact coating.

## ACKNOWLEDGMENTS

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Table 1 — Composition and Thickness of Coating Systems on Cold Rolled Steel.

Coating	A	B	C	D	E	F	G
Primer	Zinc <sup>a</sup>	Zinc <sup>a</sup>	Phosphate <sup>d</sup>	Phosphate <sup>d</sup>	IVD-Al <sup>e</sup>	IVD-Al <sup>e</sup>	Epoxy <sup>f</sup>
Midcoat		Epoxy <sup>b</sup>		Epoxy <sup>b</sup>		Epoxy <sup>b</sup>	Epoxy <sup>f</sup>
Topcoat	Epoxy <sup>b</sup>	Polyurethane <sup>c</sup>	Epoxy <sup>b</sup>	Polyurethane <sup>c</sup>	Epoxy <sup>b</sup>	Polyurethane <sup>c</sup>	Epoxy <sup>f</sup>
Avg. Thickness (μm)	53	83	43	76	81	81	81

<sup>a</sup> Zinc plate per QQ-Z-325 Rev. C (Primer Type II, Class 2).

<sup>b</sup> 2 coats of epoxy polyamide MIL-P-233377 Rev. G (Type I, Class C) per MIL-F-18264 REV. D AMD.1.

<sup>c</sup> 1 coat of MIL-C-85285 REV. B (AS) AMD.AMD.1.(AR)2 polyurethane per MIL-F-18264REV. D AMD.1 color #36375 lusterless grey of FED-STD-595.

<sup>d</sup> Phosphate coat DOD-P-16232 REV. F (Type Z, Class 3); hydrogen embrittlement relieved for 8 minimum hours at 375°F ±25°F.

<sup>e</sup> Ion vapor deposited-aluminum per MIL-C-83488-C (Notice 1, Type II, Class 1).

<sup>f</sup> 1 coat of MIL-P-24441/2 REV. B epoxy polyamide.

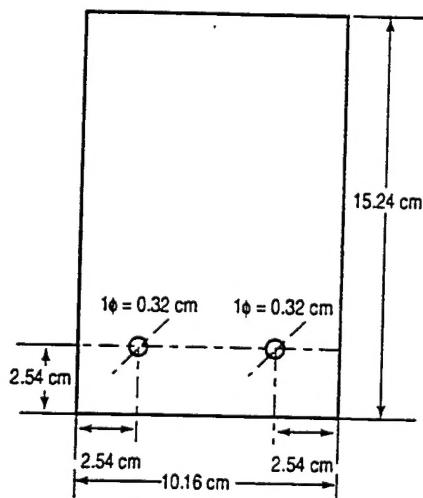


FIGURE 1 — Schematic of panel with intentional defects.

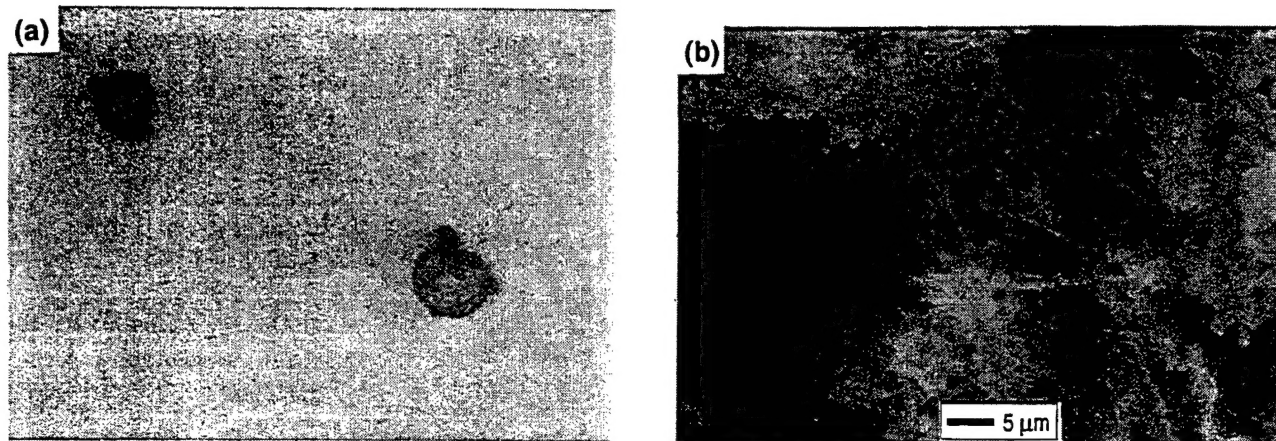


FIGURE 2 — (a) Localized corrosion on panel coated with phosphate primer and epoxy polyamide topcoat (coating C) and (b) bacteria associated with corrosion products.

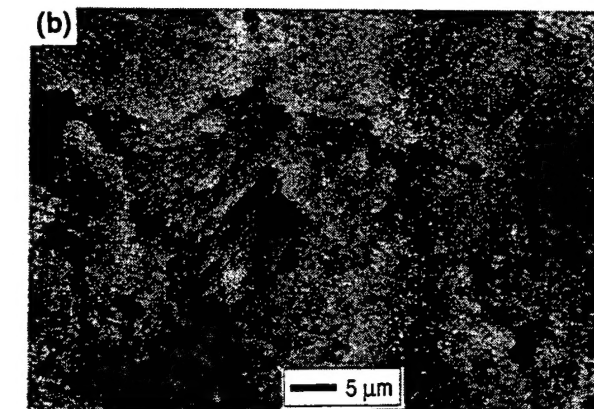
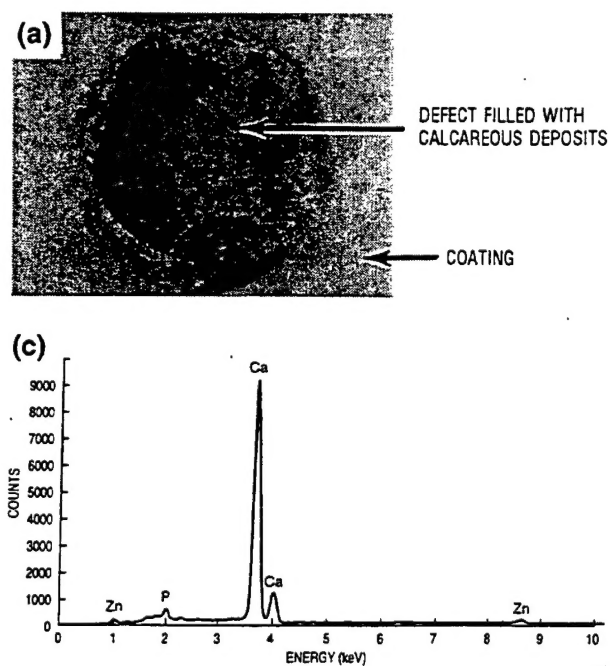


FIGURE 3 — Zinc primer, epoxy polyamide midcoat, and polyurethane topcoat (coating B): (a) calcareous deposits in defect, (b) ESEM micrograph of calcareous deposits, and (c) EDS spectrum of deposits.

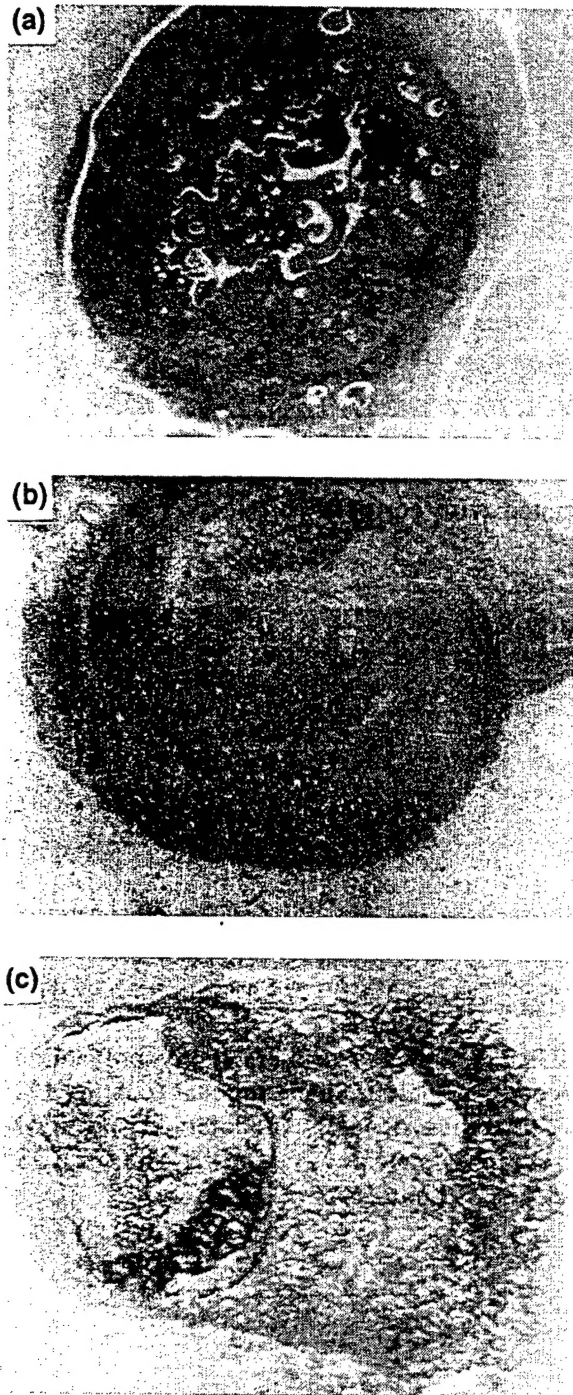


FIGURE 4 — Corrosion products in coating defects, (a) phosphate primer, epoxy polyamide topcoat (coating C), (b) phosphate primer, epoxy polyamide midcoat, polyurethane topcoat (coating D), and (c) three layers of epoxy polyamide (coating G).

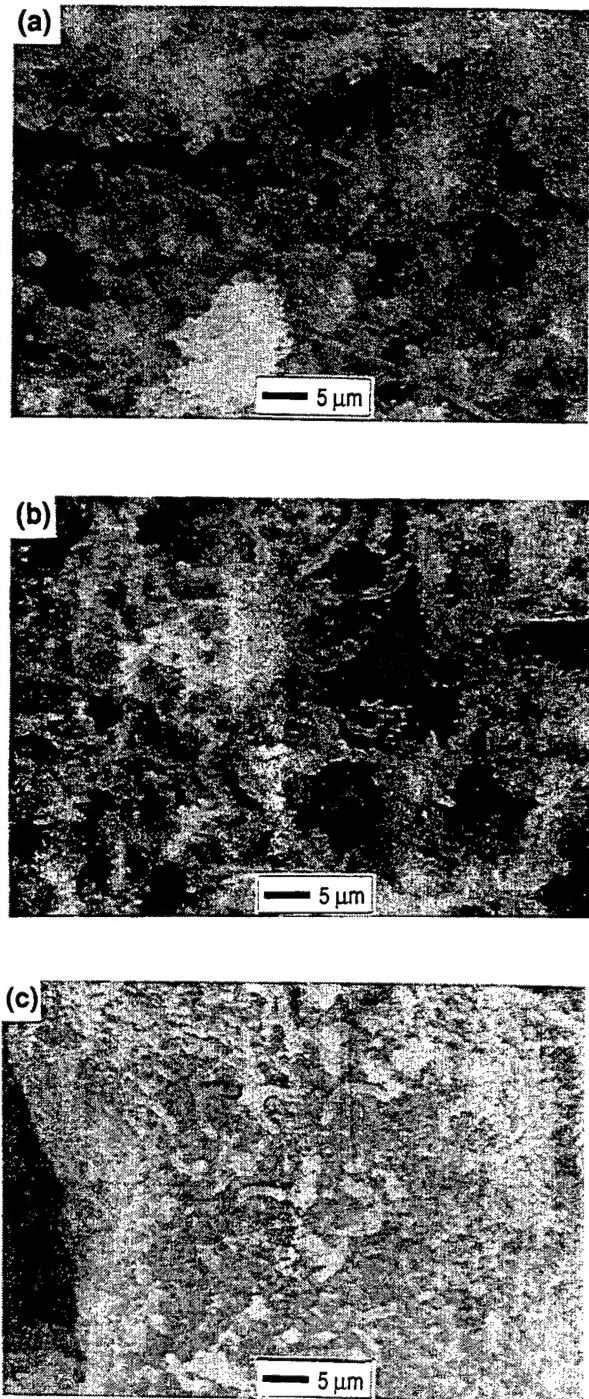


FIGURE 5 — Bacteria associated with corrosion products, (a) phosphate primer, epoxy polyamide topcoat (coating C), (b) phosphate primer, epoxy polyamide midcoat, polyurethane topcoat (coating D), and (c) three layers of epoxy polyamide (coating G).